# Phagocytes Get Close to Their Enemies

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**Phagocytosis is key for many organismal functions. In a recent issue of *Cell*, Freeman et al. (2016) demonstrate a feed-forward signaling mechanism wherein F-actin and integrin receptors drive contact formation between phagocytes and antibody-coated solid particles, signaling their engulfment. This mechanism translates nanoscale proximity effects into wider self-propagating signals.**

The challenge for innate immunity is to be able to respond to the wide variety of microbial invaders seeking to enter the host by engulfing and then killing them. While some microbes are immediately recognized via so-called "pattern-recognition" receptors, others are perceived indirectly through their opsonization with immunoglobulin G (IgG) molecules generated in the course of previous encounters with the pathogen. Receptors that bind the Fc region of IgG (FcR; Figure 1A) on phagocytes are linked to signaling subunits with cytoplasmic tyrosine motifs that are phosphorylated by Src family kinases (SFKs) and recruit Syk family tyrosine kinases, initiating phagocytosis. These kinase cascades operate on a local and quantitative balance of kinase and phosphatase activities. One of the major tyrosine phosphatases expressed by immune cells, CD45, has a variable but generally large extracellular domain, and interactions of T cell antigen receptors (TCRs) and MHC-peptide complexes require formation of a close adhesion zone that excludes CD45 (reviewed in Davis and van der Merwe, 2006). In a recent issue of *Cell*, Freeman et al. (2016) now show that CD45 exclusion is a necessary requirement for FcR-mediated phagocytosis and reveal an unexpected route to CD45 exclusion, one involving the inside-out activation of integrins and formation of a specialized type of F-actin network that together form a diffusion barrier to CD45.

To probe the mechanism of CD45 exclusion, Freeman et al. (2016) performed single-molecule tracking experiments with Quantum Dot-tagged CD45 on phagocytes interacting with IgG micro-printed onto glass substrates in 2 μm circular spots. They showed that the IgG spots establish a diffusion barrier that excluded 80%–90% of CD45 molecules that would have otherwise entered these regions, based on simulated random trajectories. The real surprise, however, was that the diffusion barrier extended well beyond (1–2 μm) the IgG spots. Further investigation revealed that the diffusion barrier corresponded to rings of integrin adhesion molecules surrounding the IgG spots. Formation of the integrin barrier was dependent upon Arp2/3-mediated actin polymerization—which can propel objects and push membranes—but not on myosin II-based contractility or longer formin-nucleated actin filaments. The strict dependence of phagocytosis on CD45 exclusion was elegantly demonstrated by showing that CD45 proteins with truncated extracellular domains that could diffuse beyond the barrier blocked phagocytosis, revealing also that the integrin-Arp2/3 system was responsible for keeping the advancing phagocytic cup very close to the opsonized particle. The ability of the integrin-Arp2/3 diffusion barrier to extend 1–2 μm beyond the site of FcR engagement was proposed to allow phagocytosis of surfaces with imperfect IgG coating, a requirement of a general phagocytic mechanism. Based on these observations, Freeman et al. (2016) proposed that FcR engagement initiates "inside-out" activation of integrins and Arp2/3-dependent actin remodeling to "squeeze" CD45 out as the phagocytic cup advances, promoting signaling (Figure 1B).

Like all important studies, the remarkable findings of Freeman et al. (2016) raise many interesting new questions. One such question relates to the nature of the diffusion barrier. Junctions formed by 15 nm adhesion complexes exclude objects as small as ~23 nm with a similar efficiency to the exclusion of CD45 observed by Freeman et al. (Alakoskela et al., 2011). Similarly, TCR interactions with MHC-peptide complexes exclude CD45 in an Arp2/3-independent fashion, whereas surrounding integrins don't exclude CD45 as effectively (Kumari et al., 2015). This can now be at least partly explained insofar as structural work shows that the smaller forms of CD45 are comparable in size to the "open" form of integrins (V.T. Chang et al., personal communication; Figure 1A). Thus, not all CD45 exclusion phenomena are Arp2/3 dependent, and not all integrin-mediated contacts create diffusion barriers. The work of Freeman et al. (2016) will nevertheless stimulate new experiments to determine whether T cells can utilize integrins for CD45 exclusion during interactions with APCs. It is also interesting to consider how relatively large integrins could facilitate FcR/IgG-driven close-contact formation in concert with Arp2/3-dependent F-actin (Figure 1B). Could Arp2/3-dependent F-actin force integrins into tertiary structures that create closer contact? Springer and colleagues proposed that lateral forces applied to the integrin β cytoplasmic domain by F-actin through talin would generate high-affinity conformations and that the lateral forces could also tilt the complexes, generating closer contacts (Zhu et al., 2008). Alternatively, F-actin-mediated protrusive forces might "break" the legs of the integrin and generate an alternative integrin configuration that could bring membranes close enough to exclude CD45 (Choi et al., 2013).

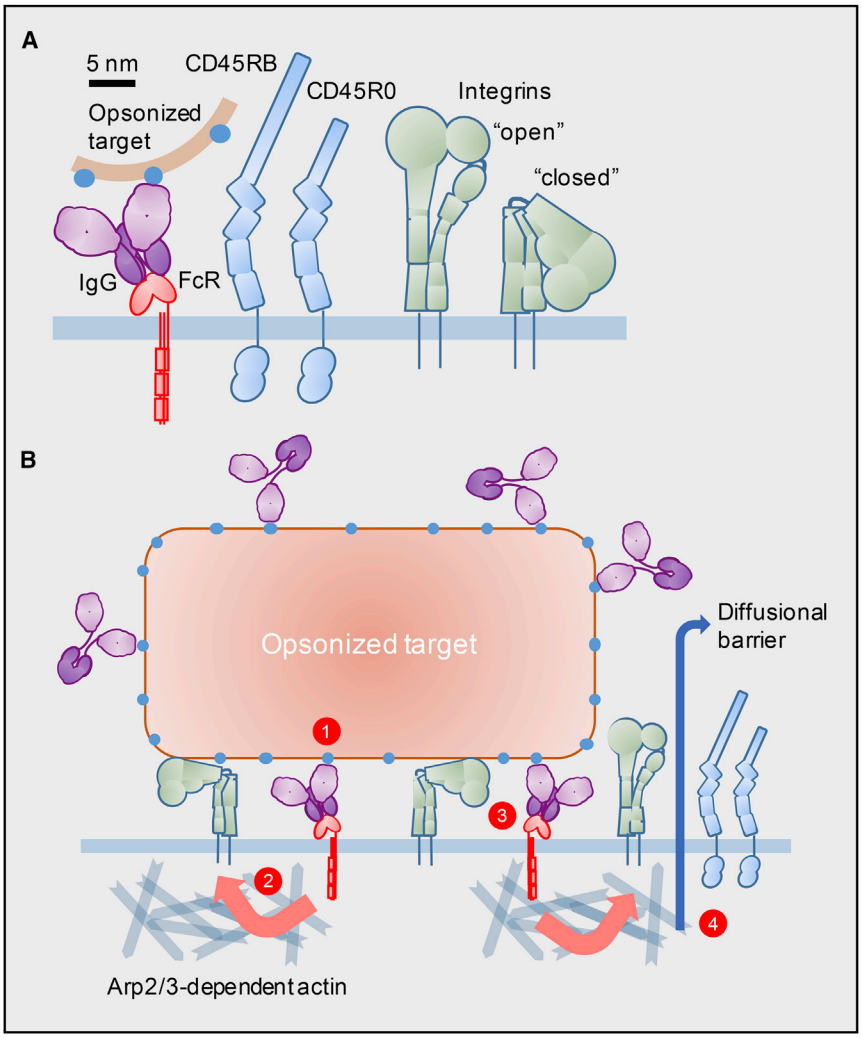


**Figure 1. Key Players in Phagocytosis**

(A) Molecules depicted and drawn to scale are FcR/IgG complexes with the horseshoe-shaped Fc region of the antibody shown in a darker color than the Fab arms (Sondermann et al., 2000), CD45R0 and modeled CD45RB (V.T. Chang et al., personal communication), and "open" and "closed" forms of integrins (Takagi et al., 2002; Xiong et al., 2001).

(B) Stages involved in phagocytic cup formation demonstrated by Freeman et al. (2016): (1) FcR engagement by IgG on an opsonized target; (2) integrin and Arp2/3-dependent actin mobilization (red arrows); (3) integrin-mediated enhancement of more FcR engagement; (4) creation by integrins of a diffusion barrier excluding CD45. In (B), the integrin is drawn in a hypothetical stooped, active form so that it fits into the gap required for FcR/IgG complex formation.

Yet another question is: at what stage(s) of phagocytosis is CD45 exclusion important? If integrin-mediated CD45 exclusion is required to promote SFK-induced signaling, what triggers the inside-out integrin activation, leading to CD45 exclusion in the first instance? One possibility is that CD45 exclusion is important at two stages and two different length-scales. FcRs have tyrosine phosphorylation motifs that make them, at least in principle, as good candidates for triggering via local, passive, size-based CD45 segregation as the TCR (Davis and van der Merwe, 2006). Thus far, however, the best evidence in favor of a purely topological mechanism of FcR triggering analogous to that proposed for the TCR is the extent to which FcRs seem to go out of their way to form very compact complexes with IgGs, first by having relatively short stalks and "bent" extracellular regions, and second, by binding to the "top" of the Fc in the middle of the IgG molecule (Figure 1A). As a consequence, FcR/IgG complexes could be expected to be very effective at topologically excluding even the smallest forms of CD45, although this would require that the IgG bind to surface-proximal targets on the microbe. Freeman et al. (2016) appear to favor an aggregation-based FcR triggering mechanism that does not require explicit phosphatase exclusion. However, if FcR/IgG complexes did initiate signaling by excluding CD45, what then would be the function of the larger-scale, integrin-dependent exclusion of CD45 to the edge of the phagocytic cup observed by the authors? One possibility is that rapid expansion of the phagocytic cup creates a high local density of CD45 ahead of it that actually enhances signaling by ensuring that SFKs are fully activated via dephosphorylation of their C termini. The integrins could in this way create a secondary wave of activated SFKs extending outward from the site of initial contact that is then able to phosphorylate substrates even more readily as soon as the diffusion barrier passes and phosphatase activity is reduced. Perhaps this is the key advantage of using a local diffusion barrier to exclude CD45, rather than other forms of sequestration: that the "excess" molecules reinforce signaling, ensuring rapid engulfment of the closely held enemy.

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